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Selected Reading

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Two Blades of the [Ex]Scissor

Boelens and coworkers (Tripsianes et al., 2005) present the structure of the heterodimeric complex of the C-terminal interaction domains of the human XPF/ERCC1 structure-specific endonuclease. The authors also provide new insights into the mechanism of XPF/ERCC1 by characterizing its DNA binding properties.

The integrity of the human genome is constantly threatened by environmental hazards such as ultraviolet (UV) radiation and chemical carcinogens, as well as endogenous toxins such as reactive oxygen species. To respond to the diverse types of DNA damage caused by these hazards, humans have developed sophisticated DNA repair mechanisms: nucleotide excision repair (NER), base excision repair, mismatch repair, and homologous recombination. Of these pathways, NER in particular repairs a broad spectrum of damage types, including bulky sunlight-induced cyclobutane pyrimidine dimers and 6-4 photoproducts.

The importance of a functional NER pathway for maintaining human health is highlighted in three hereditary autosomal recessive diseases: xeroderma pigmentosum (XP), cockayne syndrome (CS), and trichothiodystrophy (TTD). These diseases occur because of abnormalities in specific NER genes and their gene products. Patients with XP suffer from extreme photosensitivity and have an approximately 1000-fold higher incidence of squamous and basal cell carcinomas of the skin (Friedberg, 2001). Patients with CS and TTD have increased photosensitivity, but with much less likelihood for cancer progression, and also suffer other neurological and developmental disorders (Friedberg, 2001; Lehmann, 2003).

NER can be organized into three fundamental steps: (1) DNA damage recognition, (2) DNA damage excision, and (3) gap-filling synthesis and ligation. In this issue of *Structure*, Boelens and coworkers (Tripsianes et al., 2005) report on new insights into the second step of the overall NER process—damage excision. The authors describe biochemical and structural data for the human XPF/ERCC1 complex, a structure-specific endonuclease that cleaves a damaged DNA strand on the 5′ side of the lesion during the damage excision step of NER.

A major challenge to improving our understanding of the molecular mechanisms of NER is determining high-resolution 3D structures of the constituent proteins and the complexes they form. While much biochemical information has accumulated about the NER process (Riedl et al., 2003), it has been more difficult to obtain structural insight into the proteins functioning in DNA repair. This circumstance certainly has been true for the XPF/Rad1/Mus81/Hef structure-specific endonuclease family, although the 2005 publication year has witnessed a breakthrough with six reports describing structural information for members of the family. Remarkably, each study has brought its own unique advancement to the understanding of DNA damage excision.

Members of the XPF/Rad1/Mus81/Hef family possess a similar domain organization: a catalytic nuclease domain required for DNA cleavage and a helix-hairpin-helix (HhH) motif implicated in protein dimerization and DNA binding. Some family members also contain a helicase domain that may modulate interactions with DNA, although the function of the helicase-like remnant in human XPF, for example, is still ambiguous. The recent advances in structural characterization of XPF/Rad1/Mus81/Hef endonucleases have centered around these functional domains: (1) crystal structure determination of the archaeal Hef nuclease domain (Nishino et al., 2003), helicase domain (Nishino et al., 2005a), and HhH domain (Nishino et al., 2005b); (2) crystal structure determination of the crenarchaeal XPF homodimer alone and bound to dsDNA (Newman et al., 2005); (3) crystal structure determination of the central domain of human ERCC1 as well as the $(HhH)_2$ domain heterodimer of human XPF-ERCC1 (Tsodikov et al., 2005); and (4) the use of NMR cross-saturation experiments to map the interaction surface between human XPF and ERCC1 $(HhH)_2$ domains (Choi et al., 2005).

Boelens and coworkers add to this flourish of XPF/Rad1/Mus81/Hef structural work by reporting the NMR solution structure of the heterodimeric complex between human XPF and ERCC1 C-terminal $(HhH)_2$ domains. The overall fold of the $(HhH)_2$ domains in human XPF/ERCC1 closely resembles that observed in other related HhH domain structures (Nishino et al., 2005b; Newman et al., 2005; Tsodikov et al., 2005), except for the observation that the second hairpin in the tandem HhH of XPF is replaced by a 3 residue β turn. The $(HhH)_2$ dimerization interface consists of largely hydrophobic residues and is anchored by two C-terminal Phe residues (XPF Phe 894, ERCC1 Phe 293) that bind into a hydrophobic pocket formed by the partner protein's HhH motif. The analysis of the dimerization interface corresponds to that reported in other work on human XPF/ERCC1 (Tsodikov et al., 2005; Choi et al., 2005).

What distinguishes the work of Tripsianes et al. (2005) from other structural investigations of human XPF/ERCC1 is the NMR chemical shift mapping of residues involved in stem-loop DNA binding, a DNA structural equivalent of the NER bubble. Perturbations of the chemical shifts of residues in the first and second hairpin motifs of ERCC1 were observed, but not to residues in the hairpin of XPF. Thus, the authors conclude that ERCC1 possesses the DNA binding activity and XPF the nuclease activity of the protein complex. Hence, combined with the recruitment of XPF, the DNA binding activity of ERCC1 serves to localize the XPF nuclease activity to the DNA lesion to allow for damage excision. Interestingly, this model differs from that proposed by Ellenberger and coworkers (Tsodikov et al., 2005), where it was observed that two molecules of ssDNA were required to saturate binding by one human XPF/ERCC1 $(HhH)_2$ heterodimer. These authors proposed that the $(HhH)_2$ domains of both XPF and ERCC1 bind to unpaired ssDNA strands of a bubble lesion. The difference in observed DNA binding functionalities for human XPF/ERCC1 $(HhH)_2$ heterodimer may be related to the different experimental conditions and DNA substrates used by the two groups.

Another critical finding of Boelens and coworkers is that the ERCC1 $(HhH)_2$ domain folds properly only in the presence of the XPF $(HhH)_2$ domain. This implies that XPF functions as a chaperone for ERCC1, and this result has a broader impact for NER research. Structural biology of NER proteins and complexes has been greatly hindered by the inability to produce intact recombinant materials at sufficient quantities. Stabilization of structural units via coexpression could well be an invaluable strategy for the study of a variety of NER complexes.

Although the power of the domain approach is clear, several challenges lie ahead before the ultimate goal of developing a mechanistic understanding of NER at the atomic level can be achieved. One critical step involves placing domains into a more complete structural context, which includes not only the tertiary structure of the intact proteins but also an appreciation of how these proteins interact with each other. There is also a critical need to integrate results from static snapshots with data that inform the dynamic transitions between structural states. Now that the first steps have been made, the stage is set for an exciting future that will lead to a fundamental understanding of the structural mechanisms of the multiprotein assemblies in NER.

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In Silico Access to the Nuclear Pore Complex

Translocation of biomolecules through the nuclear pore complex is governed by interactions that occur between phenylalanine-glycine-rich nucleoporins and transport receptors. Using molecular dynamics simulations, Isgro and Schulten (2005) replicate and predict these interactions with startling spatial clarity and temporal detail.

The concerted interplay of biomolecules drives elementary biological processes with a spatial and temporal complexity that continues to elude the most advanced experimental techniques in biology. However, this may